

Some Serum Cytokines in Celiac Disease, Non-Celiac

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Abstract:

Back ground and aims: In This study measuring the levels of some cytokines in Najaf patients with CD, NCGS and to compare with subjects.

Methods: This case control study was done to check the levels of markers in the serum of (60) patients the celiac disease (CD), NCGS (20) and who randomly recruited from different Hospital in Najaf Iraq during period between September 2022 and January 2023 This was done using the ELISA method, age range of 40 healthy people were included this study with the same age and sex.

Results: the mean level of Interleukin -2(IL-2) high significant different between patients and healthy groups but (IL-6 and IFN γ) no significant differences, Anti-tissue transglutaminase levels were showed significant correlation an with IL-2 cytokines.

Conclusion: IL-2 play role of pathology of celiac disease.

Key words: celiac Disease, Anti-tissue transglutaminase IL-2, IL(6) IFN γ .

Introduction

Celiac disease (CD) is characterized by chronic intestinal inflammation caused by an abnormal immune response to prolamins found in wheat and other cereals(1).

The disease results from a complex interplay between genetic, environmental and immune factors leading to an inappropriate mucosal T cell response to gluten and eventually to a remodeling of the small intestinal mucosa (villous atrophy) and its clinical consequences ,The disease results from a complex interplay between genetic, environmental and immune factors leading to an inappropriate mucosal T cell response to gluten and eventually to a remodeling of the small intestinal mucosa (villous atrophy) and its clinical consequences (2).

The epidemiology of CD is well known, with an estimated worldwide prevalence of 0.6%-1% of the general population(3). However, CD remains largely underdiagnosed in developing countries and has a higher impact on children (4,5). Consequently, a new disorder known as non-celiac gluten sensitivity

(NCGS) has emerged to describe this increasingly frequent presentation to gastroenterologists and primary care practitioners (6,7).

Similar to CD, non-celiac gluten sensitivity (NCGS) as a condition, mainly associated with the ingestion of foods containing rye, barley, and wheat, can manifest various subjective symptoms, including abdominal pain, numbness in extremities, headache, and fatigue, as well as more severe neurological symptoms such as cerebellar ataxia and schizophrenia (8).

the exact definition for NCGS remains a controversial issue, and many symptoms in patients without CD and wheat allergy in response to gluten-free diet (GFD) have been considered to be associated with NCGS.

At present, there are no specific diagnostic markers for NCGS. The exclusion of CD by a negative CD serological marker and normal histology(9).

The prevalence of non- celiac gluten sensitivity largely varies among studies fluctuating between 0.16% and 13%. The lack of diagnostic biomarkers makes difficult such estimation (10).

Serological identification of autoantibodies against tissue transglutaminases (anti-tTG), gliadin (AGA) and other targets are considered as non-invasive effective methods of detection (11).

Cytokines have been extensively studied in coeliac disease, but cytokine release related to exposure to gluten and associated symptoms has only recently been described. Prominent, early elevations in serum interleukin (IL)-2 after gluten support a central role for T cell activation in the clinical reactions to gluten in coeliac disease(12).

It has been well established that CD results from an unwanted T-cell mediated autoimmune reaction. In this reaction, when the modified gliadin-derived peptides are presented through human leukocyte antigen (HLA) molecules DQ2 and DQ8 to CD4+ T helper cells, resulting from the enzymatic activity of tissue tTG, T cells and B lymphocytes get activated, producing anti-gluten and anti-tTG antibodies in lamina propria, which inevitably leads to the activation of autoreactive Th cell(13).

The activation of these autoreactive T h cells leads to the release of proinflammatory cytokines, which in turn activate intraepithelial lymphocytes, thus triggering histological alterations of the small intestinal mucosa.

Cytokines involved CD and NCGS for a better understanding of the systemic involvement of inflammatory responses in both diseases and for a possible distinguishing diagnosis tool for NCGS.

Cytokines and chemokines are key players in the immunopathology of CD.

It is important to realize that the key driving cytokines or chemokines that are involved in disease initiation, maintenance, and/or progression may not be detectable in blood. It is possible that these biomarkers are produced in narrow windows of time or they might be diluted to undetectable levels in circulation. Nevertheless, some of these proteins are relevant for diagnostics because they reveal specific signature changes in CD that can highlight different stages of disease progression (14) Interleukin-6 (IL-6) very important in promoting Th17 differentiation and have a role in several inflammatory and immune conditions as well as in CD.

Increased serum levels of IL-6 were found in patients with CD in some studies (15).

1.2. the aim of this study

1. In This study using some immunological test to diagnosis Celiac and Non celiac gluten sensitive
2. measuring the levels of some cytokines in Iraq Najaf patients with CD and NCGS and to compare with healthy subjects

Materials and method;

(60)serum samples are collected from CD patients, NCGS (20) and 40 healthy subjects a control group. Tissue transglutaminase (TTG) IgG, IGA serological antibody tests are diagnosed to all patients and the control group. Serum was isolated from peripheral blood collected from patients after informed consent All specimens are obtained from different hospitals in Najaf Iraq.

Determination the serum levels of Anti TTG, Intrelukin-2,and IFN γ : The SUNLONG Biotech Human IL-2 &IL-6 By strictly following the manufacturer's procedure, ELISA kits from Bioassay Technology Laboratory (China) were used to quantitatively assess the levels of" IL-2 & IL-6, IFN γ " in the blood of the test and control participants.

Aeskulisa Germany, SunLong Biotech, china.

Statistical analysis:

Statistical Package for the Social Science, (SPSS) program was used for data entry and analysis. All data were summarized as the mean, standard deviation of the mean, and percentage. Categorical variables were expressed as absolute numbers and percentages (%), and continuous variables were defined as means \pm standard deviation.

Results**distribute of study groups**

The ages of the people in the case group ranged from (18 -50) of celiac disease according the age(18-21) years percentages (2.50%) while range age 21-30 (46.7%), age 31-40 (38.3%), > 40(10.0).

There no significant statistical difference between age groups of patients with compared(CD, NCGS) control group as show in table (4.1)the distribution of the groups based of the sex male (31.7%)while female (65.0%) the no significant statistical differences between sex groups of patients and control.

Table(1): Difference in Age group and gender among study groups

Variables			Study Groups (n=120)			Total	P value
			Celiac Disease n=60	Gluten sensitivity n=20	Healthy control n=40		
Age group (years)	< 21	Count	3	4	4	11	0.542
		%	5.0%	20.0%	10.0%	9.2%	
	21-30	Count	28	6	15	49	
		%	46.7%	30.0%	37.5%	40.8%	
	31-40	Count	23	8	17	48	
		%	38.3%	40.0%	42.5%	40.0%	
	> 40	Count	6	2	4	12	
		%	10.0%	10.0%	10.0%	10.0%	
Total		Count	60	20	40	120	
		%	100.0%	100.0%	100.0%	100.0%	
Gender	Male	Count	19	9	17	45	0.483
		%	31.7%	45.0%	42.5%	37.5%	
	Female	Count	39	11	23	73	
		%	65.0%	55.0%	57.5%	60.8%	
	22	Count	2	0	0	2	

		%	3.3%	0.0%	0.0%	1.7%	
Total	Count		60	20	40	120	
	%		100.0%	100.0%	100.0%	100.0%	

Difference in HB, anti-t TG IgG, IL-2, IL-6 and IFN Gamma among study.

The mean number for Haemoglobin (HB) in the group of patients CD with Anemia was (11.810)g/dl and non –celiac gluten sensitivity was(12.495g/dl) the but healthy group, the mean value was(13.168) g/dl results display significant statistical difference between the patients and healthy groups ($p=0.0001$).

Serum levels of anti-tTG IgG in individuals and controls were measured.

There were important differences in mean serum concertation of Anti - t TG sera of patients groups compared with AHC(table 4.3) In our the study, the mean \pm standard deviation of Anti-t TG was(143.785 \pm 45.909 U/mL) in the CD group while in non- celiac gluten sensitivity was (29.406 \pm 19.773U/mL)

In the control group was (39.422 \pm 35.710U/mL) with a mean difference. there were When it came to measuring anti-tTG, there a significant difference between the CD, NCGS groups and the healthy group ($p=0.001$):

serum levels interleukin-2 the study groups

the Serum level IL-2 in both the CD(27.057) , NCGS(11,346)and control group(15.111). there were statistically significant differences groups ($p=0.041$)there were highly among study groups.

serum result levels IL-6 in the participants

The elevated level of IL-6 in patients with celiac disease (17.457 \pm 14.225) and non - celiac(16.061 \pm 6.249)compared with control (14.529 \pm 5.168) but there were a no significant differences as be of them table(2).

serum result levels IFN Gamma in the participants

Data showed elevated level of IFN Gamma in patients with celiac disease (46.784 \pm 80.747) and non-celiac(30.647 \pm 13.948) comparably to control (32.579 \pm 13.964) with a no significant differences table

Table(2): Difference in HB, anti-t TG IgG, IL-2, IL-6 and IFN Gamma among study groups

Markers" estimate		Study groups (N=120)			P value
		Celiac disease n=60	Gluten sensitivity n=20	Normal Control n=40	
HB g/l	Mean	11.810	12.495	13.168	0.0001
	\pm SD	1.502	1.006	1.085	
anti-t TG IgG	Mean	143.785	29.406	39.422	0.0001*
	\pm SD	45.909	19.773	35.710	
IL-2	Mean	27.057	11.346	15.111	0.041*
	\pm SD	22.428	3.322	7.682	
IL-6	Mean	17.457	16.061	14.529	0.377
	\pm SD	14.225	6.249	5.168	
IFN Gamma	Mean	46.784	30.647	32.579	0.435*
	\pm SD	80.747	13.948	13.964	

*Kruskal Wallies test was applied for comparing nonparametric data for gluten sensitivity group.

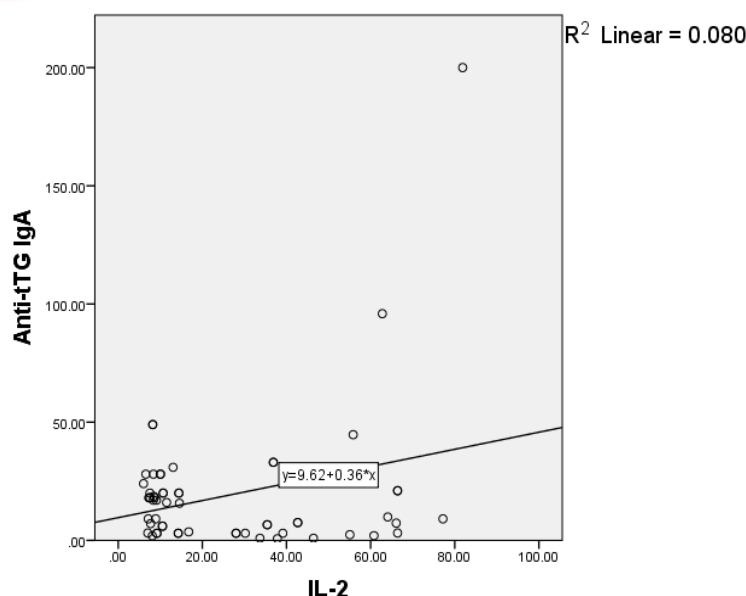
Correlation between serological marker in celiac disease patients

Patients with positivity of antibodies (Anti t TG IgA) showed the positive correlation of interleukin-2 (IL-2) was significant at the (0.05)

while IL-2 negative correlation in other antibodies (anti-t TG IgG) while patients negative correlation of IL-6 and IFN-Gamma.

Table(3): Correlation between serological marker in celiac disease patients

Serological parameter	N	Pearson Correlation	p-value
Anti-tTG IgA	IL-2	0.283	0.014*
	IL-6	-0.075	0.284
	IFN-Gamma	-0.095	0.236
anti-tTG IgG	IL-2	-0.027	0.419
	IL-6	-0.041	0.378
	IFN-Gamma	-0.028	0.416



Pearson Correlation = 0.283, p-value = 0.014*

Positive Correlation is significant.

Figure (1): is a scatter plot showing the relationship between Anti tTG-IgA and IL-2 in people with celiac disease.

people Patients with positivity of both antibodies (Anti t TG IgA and Anti -t TG-IgG) showed the positive correlation is significant of elevated IFN- Gamma As illustrated in the figures (4.9),(4.10).but IFN-Gamm is a negative correlation in others antibodies (Anti-AGA-IgA and AGA-IgG)

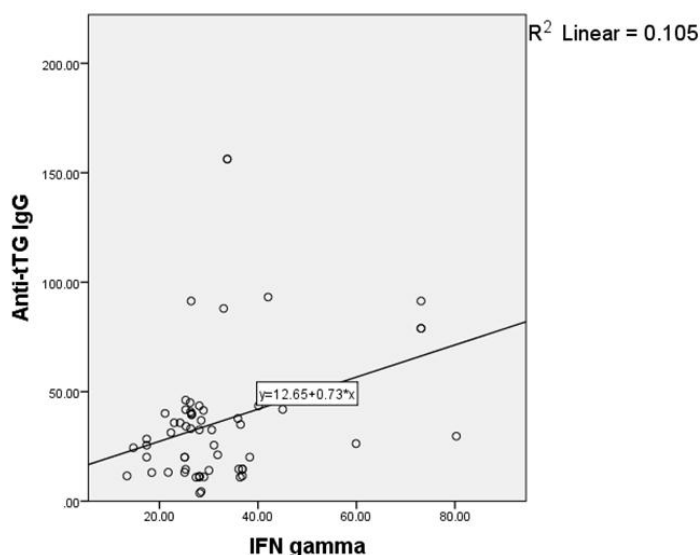
while IL-2 was a negative correlation with significant value in antibodies (anti-t TG IgG) while Negative correlation not significant differences in antibodies (Anti-t TG IgA),

while patients negative correlation of the IL-6 in

Both of antibodies (anti-t TG IgA, anti-t TG IgG,)

Table (4); Correlation between serological marker in non-celiac disease

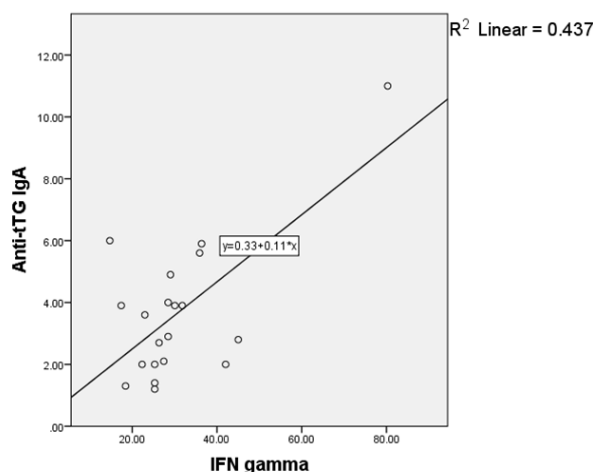
Serological parameter		N	Pearson Correlation	p-value
Anti-tTG IgA	IL-2	20	0.158	0.253
	IL-6	20	0.269	0.126
	IFN-Gamma	20	0.661	0.001**
anti-tTG IgG	IL-2	60	-0.237	0.034*
	IL-6	60	0.065	0.311
	IFN-Gamma	60	0.323	0.006**



Pearson Correlation= 0.323, $p = 0.006$

**Positive Correlation is significant at the 0.01 level (1-tailed).

Figure(2): scatter plot of the correlation between Anti-tTG IgG and IFN gamma in non-celiac disease patients



Pearson Correlation= 0.661, $p = 0.001$

**Positive Correlation is significant

Figure(3): scatter plot the correlation between Anti-tTG IgA and IFN gamma in non-celiac disease patients

Discussion

This age the study CD patients , NCGS and healthy groups were the range age from (18-50)years, **The mean age of the studied groups did not differ statistically significantly,**

Celiac disease has no specific age related group .It can appear at any age "If somebody tested negative for celiac disease at age 50, may probably he will develops symptoms at age65, because the develop gluten intolerance occurs at any age. (16).

Similar results were found (17) who reported that no significant differences be patients and AHC .

People patients with they were to gluten sensitive had an average age of (ages ranged from 18 to 75) and 79% were women. About 5% of New Zealand's to avoid gluten, though many of these instances lacked any supporting documentation for NCGS(18).

The data of study presents no significant difference between the CD NCGS and healthy groups based on gender support study(19).

These study show that most of patients had anemia a significant difference between the groups .

(20) showed195 adult patients in who Turkey who were diagnosed with CD, Anemia was found in 60.5% of them (IDA in 53.3%, folic acid deficiency in 38.4%, vitamin B12 deficiency in 25.6%, and ACD in 10.2%). These result support my study.

Anemia in NCGS may be caused by changes in the ultrastructure and molecules of enterocytes, since duodenal biopsies from people with NCGS don't show the usual histological changes of CD (21).

the serum level of IL-2 was significantly elevated patients group compared with AHC this result explained in relation to the study of (22)

who sand similar studies IL-2 release with systemic administration of gluten peptides that activates gluten –specific CD4 t cells and also placebo controlled feeding studies , the sensitivity of IL-2 release after gluten challenge is likely to be over 905% in true positive on a GFD.

Further among patients a patient is required to be adherent with a GFD before gluten causes detectable IL-2 elevations in blood needs to be clarified.

In the present study CD patients on a GFD for as little as 5 week were IL-2 responders after challenge.

Studies should also address if IL-2 assessment with gluten challenge might be a definitive test for CD when compared to histology.

Our results suggest that gluten challenge with IL-2 testing could be used to fix mistaken CD, which may happen a lot in "biopsy-confirmed" cases without supporting serology or in cases where serology alone was used to make the diagnosis. (23).

there were significant differences tested IL-6among studied groups table (4.3). probably the

Age and small sample size in study. has been shown that serum level of IL-6 was substantially elevated in patients with CD and positively correlated with inflammatory activity of disease(24).

In this study, the results also showed that IFN- not risk of CD, but its value was not different between people with CD, NCGS, and healthy people .IFN-, Innate and adaptive defence against viral, bacterial, and protozoal infections depend on type II interferon, a cytokine (25).

Some research support study such as (26).

IFN- makes the risk of CD go up, but its value as a risk of CD was very low and not significant. In our research, the IFN- level is higher in the CD group than in the healthy group(27)

In this study positivity of antibodies (Anti t TG IgA) showed the positive correlation of interleukin-2 (IL-2) is significant while IL-2 negative correlation is significant in AGA-IgA.

while IL-2 negative correlation in other antibodies(anti-t TG IgG)

while patients negative correlation of IL-6 and IFN-Gamma.

Both of antibodies(anti-t TG IgA, anti-t TG IgG,)

It was examined in other investigations if the levels of the cytokines under research are related to serum anti-TTG IgA concentrations. With the exception of INF-gamma, we observed statistically significant positive correlations between the concentrations of anti-TTG IgA and all of the cytokines..– (28)

Many studies show that IgA isotypes make up the majority of the elevated serum immunoglobulins against TTG-antibodies in CD. (29,30).

Except for IFN-gamma, our results suggest the involvement of several cytokines in the induction or maintenance of the humoral response in CD. It is not unexpected that the latter two cytokines did not exhibit a correlation with serum antibody titers given that anti-TTG IgA antibodies are the end result of a humoral response. Compared to people with less advanced histologic abnormalities, serum antibodies are more capable of accurately diagnosing people with severe cases of villous atrophy (31).

Cytokines encourage the development of IgA-producing plasma cells at the mucosal level (32).

Our results suggest that some cytokines, with the with the exception of IFN-gamma, may play a part in causing or keeping the humoral response in CD, even though tests done at a specific time cannot tell how much inflammation or systemic involvement there is. Since anti-TTG IgA antibodies are the result of a humoral reaction, it doesn't come as a surprise that the last two cytokines didn't show any correlation with serum antibody titers

Conclusions

Depending on the results obtained from this study, several conclusions can be briefly listed below:

1. IL-2 of patients are significantly higher as compared to the control group.
2. IL-6 and IFN Gamma not significant deferent between patients and control group.
3. There is a significant correlation between Anti-t TG IgA withIL-2 in celiac disease.

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